

REMARKS

The specification has been amended to correct clerical errors. Support for these amendments can be found in Fig. 11 (right hand side): construction diagram of plasmid pUAL1, restriction enzymes used are specified as "PvuII/EcoRI"; and, in SEQ ID NO:16, sequence "gaattc" corresponds to the recognition site of EcoRI. Therefore, the requested changes do not introduce new matter into the specification

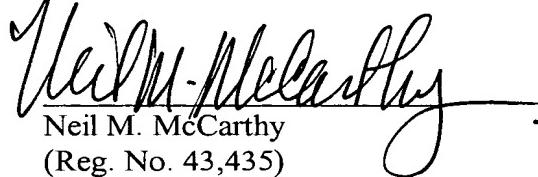
Attached hereto is a marked-up version of the changes made to the specification. The attached pages are captioned "**Version with markings to show changes made.**"

Examination in light of these amendments is respectfully requested.

The Office is hereby authorized to charge any additional fees or credit any overpayments under 37 C.F.R. §1.16 or §1.17 to Deposit Account No. 11-0600. The Examiner is invited to contact the undersigned at 202-220-4247 to discuss any matter regarding this application.

Respectfully submitted,

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Version with Markings to Show Changes Made

In the Specification:

Please replace the paragraph beginning at page 14, line 9 and ending at page 14, line 24 with the following replacement paragraph:

As to the promoter region, ALK1p with [Sall] PvuII at 5'-end and [NdeI] EcoRI at 3'-end can be prepared from SEQ ID NO:15 and NO:16 using SEQ ID NO:6 as a template. As to the terminator region, ALK1t with HindIII at 5'-end and EcoRV at 3'-end can be prepared from SEQ ID NO:17 and NO:18 using SEQ ID NO:7 as template. As to the vector, the vector pUTA1 (Fig. 2) prepared by modifying the marker gene from Ura3 to Ade1 using pUTU1 and Candida maltosa Ade1 gene (SEQ ID NO:21, GenBank D00855) [S. Kawai et al., Agric. Biol. Chem., Vol. 55, 59-65 (1991)]. By ligating ALK1p to the PvuII, [NdeI] EcoRI site of pUCNT (described in WO 94/03613) and ALK1t to the HindIII, SspI site of the pUCNT, pUAL1 (Fig 6) can be constructed. Then, by ligating ORF2 to the NdeI, PstI site of pUAL1, the plasmid pUAL-ORF2 (Fig. 7) can be constructed. Further, by ligating ORF3 to the NdeI, HindIII site of pUCNT-ALK1t in the course of construction of pUAL1 and further ligating ALK1p, pUAL-ORF3 (Fig. 8) can be constructed.

Please replace the paragraph beginning at page 21, line 25 and ending at page 22, line 15 with the following replacement paragraph:

In order that said ORF2 and ORF3 could be expressed in Candida maltosa, the Candida maltosa Alk1 gene promoter ALK1p (SEQ ID NO:6, GenBank D00481) was ligated upstream of the 5'-end of each gene and the Candida maltosa Alk1 gene terminator ALK1t (SEQ ID NO:7) was ligated downstream of the 3'-end. PCR was used for the preparation of the restriction enzyme sites necessary for ligating the promoter and terminator to the structural gene. The primer sequences used for PCR are shown in SEQ ID NO:15 through NO:18. The PCR was carried out in 25 cycles of 94 °C × 1 min., 55 °C × 2 min., 72 °C × 3 min. for amplification of the particular gene fragment. The polymerase used was Takara Shuzo's ExTaq. As to the promoter region, ALK1p with [Sall] PvuII at 5'-end and [NdeI] EcoRI at 3'-end was prepared from SEQ ID NO:15 and NO:16 using SEQ ID No:6 as a template. As to the terminator region, ALK1t with Hind III at 5'-end and EcoRV at 3'-end

was prepared from SEQ ID NO:17 and NO:18 using SEQ ID NO:7 as a template. Finally, as the vectors to which ORF2 and ORF3 were to be ligated, there were used pUTU [M. Ohkuma et al., J. Biol. Chem., Vol. 273, 3948-3953 (1998)] which is obtained by ligating the autonomously replicating sequence (ARS) of Candida maltosa (GenBank D29758) and URA3 gene (GenBank D12720) to pUC19 and pUTA1 (Fig. 2) which is obtained by modifying the marker gene from Ura3 to Ade1 using Candida maltosa ADE1 gene (SEQ ID NO:21; GenBank D00855). pUTA1 was constructed by removing URA3 gene from pUTU1 with Xhol and ligating the ADE1 gene fragment as excised with Sall.

Please replace the paragraph beginning at page 22, line 16 and ending at page 22, line 23 with the following replacement paragraph:

ALK1p was ligated to the PvuII, [NdeI] EcoRI site of pUCNT (described in WO94/03613) and ALK1t was ligated to the HindIII, SspI site of the pUCNT to construct pUAL1 (Fig. 6). Then, ORF2 was ligated to the NdeI, PstI site of pUAL1 to construct a plasmid pUAL-ORF2 (Fig. 7). Moreover, in the course of construction of pUAL1, ORF3 was ligated to the NdeI, HindIII site of pUCNT-ALK1 and, further, ALK1p was ligated to construct pUAL-ORF3 (Fig. 8).